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Peels extract of *Punica Granatum*, *Citrus Limetta* and *Musa Paradisiaca* and it's antioxidant efficacy

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ABSTRACT

The primary supply of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds, and other nutrients in the globe is found in fruits and fruit peels. They are a source of supplementary antioxidants as well. DPPH radical scavenging, Catalase activity, and Total Reducing Power were used to assess the antioxidant potential of Punica granatum, Citrus limetta, and Musa paradisiaca. The best antioxidant activity for DPPH assays is demonstrated by methanol peel extracts of Punica granatum (97.35), Musa paradisiaca (98.1), and Citrus limetta (96.08) at a concentration of 0.1 mM/ml. Citrus limetta and Musa paradisiaca had the highest antioxidant capabilities, with catalase activity of fresh Punica granatum peel measuring 0.13 mg/ml, 0.02 mg/ml, and 0.02 mg/ml, respectively. The greatest total decreasing inhibition. Citrus limetta (98.35%), Musa paradisiaca (97.53%), and Punica granatum peel extract have the highest levels of overall reducing power inhibition. The antioxidant efficiency of each fruit peel extract used in the current study is good.

1. Introduction

A healthy diet should include fruits. They are regarded as a modest primary antioxidant source but a potent secondary antioxidant source.[1] Fruits and fruit peels are one of the most important food crops in the world and a fantastic source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds, and other nutrients.[2,3] The polyphenols found in fruit skins or peels are the most frequently targeted substances.[4] It is not advised to extract these compounds at high temperatures since they become unstable at those temperatures, which greatly diminishes their concentration levels. An antioxidant is a chemical that prevents other molecules in the biological system from oxidising. A chemical reaction called oxidation involves the transfer of electrons or hydrogen from a material to an oxidising agent. Free radicals can be produced through oxidation processes. The antioxidants found in fruits and fruit waste, such as ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins, are thought to play a significant role in the prevention of these diseases.[5,6] In turn, these radicals can start chain reactions, when the chain reaction occurs in a cell (biological system).[7] Extraction techniques typically involve certain pre-treatment and post-treatment procedures in order to boost the yield of bioactive chemicals and lower the amount of solvents and energy consumed.[8] Polyphenols and polysaccharides interact non-covalently, making them resistant to extraction by polar solvents.[9] The yield of anthocyanins from wine lees was doubled when we performed conventional solid-liquid extraction followed by microwave (MW) and ultrasound (US) pre-treatments.[10] The highly reactive molecule oxygen can combine with other molecules to form potentially harmful ones known as "free radicals," which are known to cause harm. The healthy cells in the body can be attacked by free radicals,

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which renders them less active in terms of structure and function.[11] Free radicals and other highly reactive oxygen-containing molecules are all referred to as reactive oxygen species, or ROS. There are several other forms of ROS molecules present here, including the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides.[12] Biomolecules including DNA, proteins, and/or lipids may suffer reversible or permanent damages as a result of free radicals.[13,14] A significant source of minerals, including potassium, calcium, phosphorus, and magnesium, the punica granatum peel makes up around 50% of the fruit's weight. About 50% of the weight of the fruit is made up of the Punica granatum peel, which is also a significant source of minerals, particularly potassium, calcium, phosphorus, magnesium, and sodium.[15] It also contains complex polysaccharides, high levels of a wide variety of bioactive compounds, including phenolics, flavonoids, proanthocyanidin compounds, and ellagitannin (ETs), such as punicalagins and its isomers, as well as lesser amounts of The main waste component is the peel and seed residue from Citrus limetta. Peels from citrus limetta, which may be combined with dried pulps to make bovine feed, are a significant source of molasses, pectin, cold-pressed oils, and limonene. Peels and seeds are both excellent sources of phenolic chemicals, including as flavonoids and phenolic acids. The polymethoxylated flavones and the glycosylated flavanones are two highly strange families of chemicals that are responsible for the presence of flavonoids in citrus fruits.[16] Musa paradisiaca are solid fruits with creamy flesh and a sweet flavour that are wrapped in their distinctive yellow jackets. One of the fruits with the highest global consumption is Musa paradisiaca.[17] In addition to carbohydrates, cellulose, and minerals like potassium and sodium, the peel of Musa paradisiaca is high in flavonoids and other phenolic compounds. Bioactive substances that function as antioxidants include flavonoids and phenol.[18,19]

2. Materials and Methods

2.1. Waste fruit collection (Peels)

Peels from pomegranate (Punica granatum), sweet lime (Citrus limetta), and banana (Musa paradisiaca) trees, as well as bananas (Musa paradisiaca), were gathered from the Shrigonda local market.

2.2. Chemicals and reagents

Methanol, potassium phosphate buffer, hydrogen peroxide, DPPH (2, 2-Diphenyl-1-picrylhydrazyl), potassium ferricyanide, tri-chloro acetic acid, and ferric chloride. (Collected from college Laboratory)

2.3. Preparation of extract

In 10ml of potassium phosphate buffer and methanol, 0.5g (500mg) of each of the peels from Punica granatum, Citrus limetta, and Musa paradisiaca were finely ground in a cooled mortar and pestle. After extraction, the homogenates samples were centrifuged at 5000 rpm for 15 minutes. The supernatants were then transferred to a new tube and used for the performance experiments. The homogenates samples were filtered using Whatman filter paper.

3. Assay of Dpph Radical Scavenging Activity

The method described by Brand Williams[20] was used to investigate the antioxidant activity of Punica granatum peel, Citrus limetta peel, and Musa paradisiaca peel extracts using 0.1mM DPPH (2,2 diphenyl-1-picryl hydrazyl) radical scavenging. In a 3 ml test tube, an aliquot of 100 l of the sample extract was combined with 2.9 ml of newly made DPPH working solution. After combining the ingredients, the reaction mixture was placed in a dark area for 30 minutes with the test tube covered with aluminium foil. Using a UV-VIS spectrophotometer, the solution's absorbance was measured at 517 nm against methanol. Equation No. 1 was used to calculate antioxidant activity, which was reported as a percentage suppression of the DPPH radical. Units/mg were used to compute the O.D values.

The formula was used as,

Radical scavenging **(%) =** [(A blank **–** A sample) **/** A blank] × 100 ..... Equation (1)

4. Assay of Catalase

The method of[21] was used to determine the catalase activity in the chosen plant samples. Keep hydrogen peroxide in the shadows. Since the reaction happens very quickly and hydrogen peroxide is light-sensitive, the contents of the test tube were transferred into the cuvette before obtaining absorbance. At 240nm, the O.D. was taken. Between 230 and 250 nm, hydrogen peroxide's absorption of UV light can be easily determined. The absorption reduces over time as a result of catalase's breakdown of hydrogen peroxide. This drop in absorption can be used to assess the enzyme activity. Units/mg of protein were used to compute the O.D values.

The formula was used as,

D = [(ΔA/min)/concentration] ×100

Where,

Activity = D/ extinction coefficient

Extinction coefficient = 3.94mM/cm

ΔA is highest O.D- lowest O.D

5. Total Reducing Power

In the reduction of Fe3+(CN)6 to Fe2+(CN)6, the direct electron donation method was used to measure the reducing

power.[22] The phosphate buffer extracts contained 2.5 ml of the fruit waste sample in varied concentrations, 2.5 ml of potassium ferricyanide at 1%, and 2.5 ml of phosphate buffer at 50 mM. The only reagent absent from the control was the sample. 20 minutes were spent heating the mixture to 50 ° C. Following the addition of 2.5 ml of 10% (w/v) tri-chloroacetic acid, the mixture was centrifuged at 5000 rpm for 10 minutes. A fresh centrifuge tube was used to transfer 5ml of the supernatant upper layer, which was then combined with 5ml of double-distilled water and 1ml of 0.1% ferric chloride. At 700 nm, the absorbance was measured in comparison to phosphate buffer and distilled water blanks. Increased absorbance implies that the extract sample has more reducing power. Units/mg of protein were used to compute the O.D values.

The formula was used as,

% inhibition **=** 100 × (A control -A sample) **/** A control

6. Result

7. Antioxidant activity of different fruit peels extract

Citrus limetta and Musa paradisiaca peel extracts were tested in DPPH (2,2 diphenyl-1-picryl hydrazyl) radical scavenging, Catalase activity(H2O2), and Total Reducing assay after Punica granatum fresh methanol peel extract and fresh phosphate buffer (PH-7.2) peel extract were evaluated for their antioxidant activity.

7.1. Antioxidant of DPPH (2, 2 diphenyl-1-picryl hydrazyl) radical scavenging

Other compounds' oxidation processes can be slowed down or stopped by antioxidants[23] The DPPH radical scavenging assay measures the drop in absorbance during oxidation processes to estimate antioxidant capability. Based on the antioxidants' ability to prevent colour loss, the scavenging activity was calculated. Visually, it appears as a shift from purple to yellow in colour. The drop in DPPH radical's absorbance at 517 nm brought on by antioxidants is used to calculate the radical's capacity for reduction.[15] The findings of this investigation revealed that there was a considerable distinction between the samples of Punica granatum, Citrus limetta, and Musa paradisiaca (Table 1). Punica granatum peel inhibition was lowest among the samples (93.940.24), followed by Citrus limetta peel (96.080.03) and Musa paradisiaca peel (97.360.03). (Figure 1). Although there was a statistically significant difference between the samples of Punica granatum, Citrus limetta, and Musa paradisiaca, the fresh peel extract was less effective at scavenging DPPH radicals.Although there was a statistically significant difference between the samples of Punica granatum, Citrus limetta, and Musa paradisiaca, the fresh peel extract was less effective at scavenging DPPH radicals.

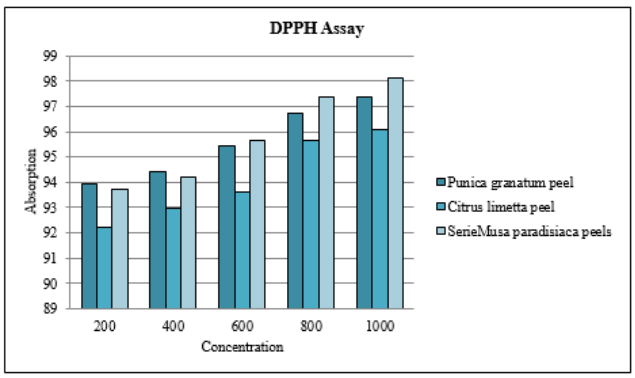

Fig. 1: Comparative studies of DPPH activity for punica granatum peel, citrus limetta peel and musa paradisiaca peel

8. Antioxidant of Catalase

By using a reducing power test, the antioxidant activity of the peels of Punica granatum, Citrus limetta, and Musa paradisiaca was determined. According to research, the production of reductones coincides with the antioxidative action. Reductones' antioxidant activity, according to Aebi 1984,[21] is dependent on the radical chain being broken by hydrogen atom donation. Free radicals are changed by antioxidants into more stable molecules that can stop a chain reaction of radicals. With increasing concentration in all solvents, the various fruit peel extracts' catalase activity increased (Table 2 & Figure 2). In comparison to Citrus limetta and Musa paradisiaca peels (0.02–0.04 Unit per Minutes per Milligram), Punica granatum peel extracts had a better lowering capability (0.13–0.04 Unit per Minutes per Milligram of Fresh Weight). Punica granatum peel extract had a higher reduction capacity than Citrus limetta and Musa paradisiaca peel extract (0.02–0.04 Unit per minutes per milligramme of fresh weight), although the differences were only really noticeable in Punica granatum peels.

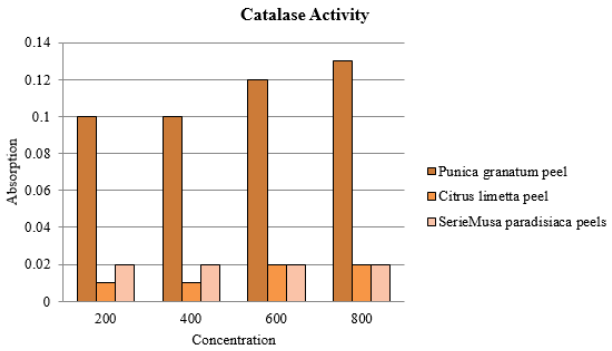

Fig. 2: Comparative studies of catalase for punica granatum peel, citrus limetta peel and musa paradisiaca peel

Table 1: DPPH Activity

Sample	DPPH Result (mg/ml) % Inhibition				
	200 μl	**400 μl**	**600 μl**	**800 μl**	**1000 μl**
Punica granatum peel	93.94±0.24	94.43±0.28	95.42±0.36	96.73±0.74	97.35±0.04
Citrus limetta peel	92.23±0.23	92.98±0.27	93.62±0.19	95.66±0.26	96.08±0.03
Musa paradisiaca peel	93.70±0.48	94.23±0.71	95.65±0.29	97.36±0.03	98.1±0.03

Table 2: Antioxidant activity of *catalase limetta paradisiaca limetta paradisiaca ion* of Fe3+ into Fe2+ in the presence of different fractions was measured.[24]Reductones, which are antioxidants that exert their antioxidant activity by donating a hydrogen atom to break the free radical chain, are often necessary for a chemical to have

Sample	**Result (U/min /mg of FW)**			
	200 μl	**400 μl**	**600 μl**	**800 μl**
Punica granatum peel	0.01±0.20	0.01±0.08	0.01±0.30	0.13±0.32
Citrus limetta peel	0.01±0.00	0.01±0.12	0.02±0.25	0.02±0.00
Musa paradisiaca peel	0.02±0.00	0.02±0.00	0.02±0.26	0.02±0.05

9. Antioxidant of Total Reducing Assay

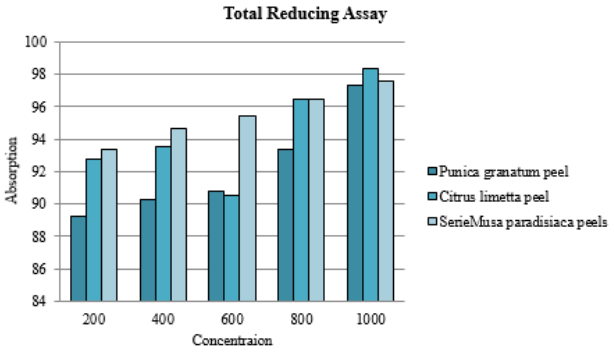

Fig. 3: Comparative studies of total reducing assay for punica granatum peel, citrus limetta peel and musa paradisiaca peel

10. Discussion

In a wide range of clinical symptoms, free radicals are known to have a clear function. Antioxidants neutralise free radicals and defend against several illnesses. In order to make use of their exploit, they either scavenge reactive oxygen species or safeguard the antioxidant defence mechanisms. The extracts' antioxidant properties led to the transformation of the Fe3+/ferric cyanide complex into ferrous form, demonstrating their reducing potential. When compared to other free radicals, the hydroxyl radical has the shortest half-life and is the most harmful and reactive of all ROS. Deoxyribose is converted to malondialdehyde by the oxygen-derived hydroxyl radicals and the additional transition metal ion (Fe2+)[14,25]. Thiobarbituric acid is then used to produce a pink chromogen. Due to its high bioavailability, vitamin C is a significant antioxidant compound as well. It works by preventing oxidative damage to the membrane and low-density lipoproteins.[3] The examined cultivars had vitamin C contents that ranged from 35.88 to 62.11 mg per 100 g, with Imperial cultivar having the greatest concentration and Vitória cultivar having the lowest.[26] During a lipid oxidation, several radical species with various levels of reactivity are created (OH, O2-, L., LOO., LO., etc.). The assessment of the antioxidant activity technique for screening individual compounds as well as various plant extracts has made extensive use of the very stable organic radical DPPH.[20,27,28] With the addition of the fractions in a concentration-dependent manner, DPPH, a stable, nitrogen-centered free radical that gives violet colour in ethanol solution, is reduced to diphenylpicryl hydrazine, a yellow result. The decrease in the amount of DPPH molecules is related to the availability of hydroxyl groups. The extracts demonstrated a considerably higher hydrogen-donating activity and maximal inhibition percentage, which were strongly correlated with total phenolic content. Comparing the results of the current study to those of Pooja Shah's,[29] the results showed better scavenging activity. At 0.1 mM/ml, the Punica granatum, Citrus limetta, and Musa paradisiaca methanol peel extracts demonstrated 93.94%, 96.08%, and 97.36% DPPH scavenging values, respectively. According to Moraes Barros et al.,[30] the peel of Citrus limetta has a higher phenolic content than the pulp, which increases its ability to scavenge DPPH radicals. Crude C. limetta juice was found to have a 261 M TE antioxidant activity, according to Barreca et al.,[31] who also reported that the juice's low flavonoid content was a contributing factor to its antioxidant activity. Citrus limetta's antioxidant activity was rated as having a high degree of activity when compared to other fruits, according to Ali et al.[32] 's analysis of the antioxidant activity of several fruits (orange, mango, lemon, papaya, etc.). Musa paradisiaca peel extracts have been shown to be able to scavenge DPPH radicals (Okonogi et al 2007).[33] As a result, the percentage of DPPH inhibition used to measure each sample's DPPH scavenging activity was reported.[34] A higher value indicates greater antioxidant activity. The hydroxyl radical is a kind of highly reactive free radical that can harm virtually every molecule

Table 3: Total reducing assay

Sample	Total Reducing assay Result (mg/ml) % Inhibition				
	200 μl	400 μl	600 μl	800 μl	1000 μl
Punica granatum peel	89.21±0.27	90.25±0.18	90.77±0.25	93.36±0.46	97.34±0.31
Citrus limetta peel	92.72±0.28	93.56±0.47	90.55±0.30	96.41±0.27	98.35±0.14
Musa paradisiaca peel	93.37±0.24	94.61±0.33	95.38±.0.31	96.46±0.13	97.53±0.30

present in living cells. It can be produced in vivo through the Haber-Weiss reaction when superoxide radicals and transition cations like iron or copper are present.[35] Using the hydroxyl radical prevention ability experiment created by Ou et al.[36] researchers were able to assess the ability of peel and pulp extracts to fend off hydroxyl radicals. The findings showed that the peel extract had approximately 25 times more activity than the pulp extract. Since the method is based on the antioxidants' ability to chelate metals, the so-called preventative capacity against hydroxyl radicals is really correlated with the samples' ability to do the same.[37] Regarding the solvents utilised for extraction, peel extracts followed a similar pattern, although pulp had a higher scavenging potential. Hexane and aqueous extracts, which included all of the peel extracts, demonstrated 8% and 11% higher activity than pulp whereas the others were equivalent. Overall, it was discovered that the scavenging potential of the fruit's constituents was comparable (F = 0.089ns). Methanol extracts produced low concentrations of 11.4 g/ml and 10.8 g/ml in the pulp and peel, respectively; 13–14 times more aqueous extracts were needed to quench 50% of the hydroxyl radical.[38] Researchers have looked at the overall decreasing power of fruit peels from Musa paradisiaca, Citrus limetta, and Punica granatum. In comparison to Punica granatum and Musa paradisiaca, Citrus limetta has reductive properties, however the activity of the extract is lower. According to Gordon, 1990,[39] the antioxidant activity of reductones is based on the radical chain being broken by the donation of one atom of hydrogen. As a result of their interaction with free radicals, antioxidants can stop a chain reaction of radicals by converting them into more stable molecules. As concentration grew, so did the components of the various fruit peel extracts' reducing power. Because pulp has a greater reducing capability than peel extracts, ascorbic acid concentration in pulp may be higher.[38]

11. Conclusion

The antioxidant activity of fruit peel extracts from Punica granatum, Citrus limetta, and Musa paradisiaca was assessed in the current study using three straightforward spectrophotometric methods: DPPH (2, 2-Diphenyl-1-Picrylhydrazyl), Catalase, and Total Reducing Assay. The peel extracts of Punica granatum, Musa paradisiaca, and Citrus limetta had the best antioxidant activity for DPPH tests when the 0.1mM/ml DPPH was dissolved in methanol and examined. Citrus limetta and Musa paradisiaca have the highest antioxidant activities as measured by catalase activity, followed by Punica granatum and Musa euphorbia. According to the results of the total reducing assay, Musa paradisiaca, Punica granatum, and Citrus limetta peel extracts had the highest antioxidant activity. The findings of all three assays showed that the level of phenolics in the peel extract of Punica granatum, Citrus limetta, and Musa paradisiaca fruit, which are high in phenolics, may be a suitable source of antioxidant.

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13. Source of Funding

None.

14. Conflict of Interest

None.

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